

AMENDMENTS TO THE CLAIMS

- Claim 1. (Withdrawn) A method of generating ascorbic acid, comprising:
culturing a *Kluyveromyces* spp. or a *Zygosaccharomyces* spp. yeast in a medium comprising an ascorbic acid precursor, thereby forming ascorbic acid, and
isolating the ascorbic acid.
- Claim 2. (Withdrawn) The method of claim 1, wherein the yeast is a *Z. bailii* or a *K. lactis*.
- Claim 3. (Withdrawn) The method of claim 2, wherein the yeast is *Z. bailii* strain ATCC 60483 or *K. lactis* strain PM6-7A.
- Claim 4. (Withdrawn) The method of claim 1, wherein the ascorbic acid precursor is L-galactose; D-glucose; L-galactono-1,4-lactone; or L-gulonono-1,4-lactone.
- Claim 5. (Withdrawn) The method of claim 1, wherein the isolating step comprises lysing the yeast.
- Claim 6. (Withdrawn) The method of claim 5, wherein the isolating step further comprises centrifugation, filtration, microfiltration, ultrafiltration, nanofiltration, liquid-liquid extraction, crystallization, enzymatic treatment with nuclease or protease, or chromatography.
- Claim 7. (Previously presented) A method of generating ascorbic acid, comprising:
- a) obtaining a recombinant yeast capable of converting an ascorbic acid precursor into ascorbic acid, wherein the yeast is functionally transformed with a coding region encoding a first enzyme selected from D-arabinose dehydrogenase (ARA), D-arabinono-1,4-lactone oxidase (ALO), or L-gulonono-1,4-lactone oxidase (RGLO),
 - b) culturing the recombinant yeast in a medium comprising an ascorbic acid precursor, thereby forming ascorbic acid, and
 - c) isolating the ascorbic acid.

Claim 8. (Original) The method of claim 7, wherein the yeast belongs to the genus *Saccharomyces*, *Zygosaccharomyces*, *Candida*, *Hansenula*, *Kluyveromyces*, *Debaromyces*, *Nadsonia*, *Lipomyces*, *Torulopsis*, *Kloeckera*, *Pichia*, *Schizosaccharomyces*, *Trigonopsis*, *Brettanomyces*, *Cryptococcus*, *Trichosporon*, *Aureobasidium*, *Lipomyces*, *Phaffia*, *Rhodotorula*, *Yarrowia*, or *Schwanniomyces*.

Claim 9. (Previously presented) The method of claim 8, wherein the yeast belongs to the species *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, or *Z. bailii*.

Claim 10. (Original) The method of claim 9, wherein the yeast is selected from *S. cerevisiae* strain GRF18U; *S. cerevisiae* strain W3031B; *K. lactis* strain PM6-7A; or *Z. bailii* strain ATCC 60483.

Claim 11. (Cancelled)

Claim 12. (Previously presented) The method of claim 7, wherein the ARA enzyme has at least about 70% similarity with SEQ ID NO:20; the ALO enzyme has at least about 70% similarity with SEQ ID NO:5 or SEQ ID NO:7; or the RGLO enzyme has at least about 70% similarity with SEQ ID NO:9.

Claim 13. (Previously presented) The method of claim 7, wherein the ARA enzyme has at least about 70% identity with SEQ ID NO:20; the ALO enzyme has at least about 70% identity with SEQ ID NO:5 or SEQ ID NO:7; or the RGLO enzyme has at least about 70% identity with SEQ ID NO:9.

Claim 14. (Previously presented) The method of claim 7, wherein the coding region encoding the ARA enzyme has at least about 70% identity with SEQ ID NO:21; the coding region encoding the ALO enzyme has at least about 70% identity with SEQ ID NO:6 or SEQ ID NO:8; or the coding region encoding the RGLO enzyme has at least about 70% identity with SEQ ID NO:10.

Claim 15. (Previously presented) The method of claim 7, wherein the yeast is functionally transformed with a coding region encoding ALO.

Claim 16. (Previously presented) The method of claim 7, wherein the yeast is functionally transformed with a coding region encoding ARA.

Claim 17. (Original) The method of claim 16, wherein the ARA comprises the amino acid sequences GXRXXDXAXXXXEXXXG (SEQ ID NO:13) and GXXN (SEQ ID NO:26).

Claim 18. (Original) The method of claim 7, wherein the coding region is linked to a promoter active in the yeast.

Claim 19. (Original) The method of claim 18, wherein the promoter is the *S. cerevisiae* triosephosphateisomerase (TPI) promoter.

Claim 20. (Previously presented) The method of claim 7, wherein the coding region was isolated from *Arabidopsis thaliana*, *S. cerevisiae*, or *Rattus norvegicus*.

Claim 21. (Currently amended) The method of claim ~~20~~ 24, wherein the coding region encoding LGDH was isolated from *A. thaliana*, the coding region encoding ALO was isolated from *S. cerevisiae*, the coding region encoding AGD was isolated from *A. thaliana*, the coding region encoding ARA was isolated from *S. cerevisiae*, or the coding region encoding RGLO was isolated from *R. norvegicus*.

Claim 22. (Currently amended) The method of claim ~~7~~ 24, wherein the AGD enzyme comprises a signaling peptide.

Claim 23. (Currently amended) The method of claim ~~7~~ 24, wherein the AGD enzyme does not comprise a signaling peptide.

Claim 24. (Currently amended) The method of claim 7, wherein the yeast is functionally transformed with a coding region encoding a second enzyme other than the first enzyme, wherein the second enzyme is selected from L-galactose dehydrogenase (LGDH), L-galactono-1,4-lactone dehydrogenase (AGD), ARA, ALO, or RGLO.

Claim 25. (Original) The method of claim 24, wherein the coding region encoding the second enzyme is linked to a promoter active in the yeast.

Claim 26. (Original) The method of claim 25, wherein the promoter is the *S. cerevisiae* triosephosphateisomerase (TPI) promoter.

Claim 27. (Previously presented) The method of claim 7, wherein the recombinant yeast further comprises at least one coding region encoding an enzyme associated with the conversion of a carbon source to L-galactose.

Claim 28. (Original) The method of claim 7, wherein the ascorbic acid precursor is selected from L-galactono-1,4-lactone; D-glucose; L-gulono-1,4-lactone; or L-galactose.

Claim 29. (Original) The method of claim 7, wherein the isolating step comprises lysing the yeast.

Claim 30. (Original) The method of claim 29, wherein the isolating step further comprises centrifugation, filtration, microfiltration, ultrafiltration, nanofiltration, liquid-liquid extraction, crystallization, enzymatic treatment with nuclease or protease, or chromatography.

Claim 31. (Original) The method of claim 7, wherein the recombinant yeast accumulates L-ascorbic acid in the medium at levels greater than background.

Claim 32. (Original) The method of claim 31, wherein the isolating step comprises chromatography, activated carbon, microfiltration, ultrafiltration, nanofiltration, liquid-liquid extraction, or crystallization.

Claim 33. (Original) The method of claim 7, wherein the recombinant yeast produces ascorbic acid with a yield greater than about 35% from a precursor.

Claim 34. (Canceled)

Claim 35. (Withdrawn) A *S. cerevisiae*, wherein the *S. cerevisiae* is functionally transformed with one or more coding regions selected from a coding region encoding D-arabinose dehydrogenase (ARA), a coding region encoding D-arabinono-1,4-lactone oxidase (ALO), a coding region encoding L-galactono-1,4-lactone dehydrogenase (AGD) and a coding region encoding L-galactose dehydrogenase (LGDH), a coding region encoding D-arabinono-1,4-lactone oxidase (ALO) and a coding region encoding L-galactose dehydrogenase (LGDH), or a coding region encoding D-arabinono-1,4-lactone oxidase (ALO) and a coding region encoding D-arabinose dehydrogenase (ARA).

Claims 36-40. (Cancelled)